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SYNTHESIS OF ADENOSINE TRIPHOSPHATE UNDER POSSIBLE PRIMITIVE EARTH CONDITIONS

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IT has been suggested that the pre-biological synthesis of nucleoside phosphates on the primitive Earth was a consequence of the absorption of ultra-violet light by purines and pyrimidines in an appropriate aqueous medium[1,2]. The basis for this suggestion is as follows:

Even the simplest living organisms are statistically unlikely aggregations of organic molecules. The improbability of contemporary organisms is extracted from the field of possibilities through natural selection. But before the advent of self-replicating systems, natural selection as we understand it to-day could have played no such part. The origin and subsequent replication of life must therefore have involved molecules preferentially produced in the primitive environment. Such a view is implicit in the early works of Haldane[3] and Oparin[4]. While it is possible that the fundamental molecular basis of living systems has itself evolved, the simplest working hypothesis holds that the molecules that are fundamental now were fundamental at the time of the origin of life. The production of amino-acids, purines, pyrimidines and pentose sugars under simulated primitive conditions during the past decade lends support to this hypothesis.

There are, however, still several molecular species the involvement of which in the origin of life remains to be demonstrated. Chief among these are the nucleoside phosphates. Adenosine triphosphate (ATP) is the 'universal' energy intermediary of contemporary terrestrial organisms, and one of the major products of plant photosynthesis. The need for its production in primitive times was first emphasized by Blum[5]. Guanosine triphosphate has recently been implicated as the energy source for peptide linkage[6]. The deoxynucleoside triphosphates are the precursors for contemporary DNA biosynthesis[7].

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To the extent that the origin of DNA plays a fundamental part in the origin of life, the abiogenic synthesis of deoxynucleoside triphosphates seems indicated[2]. Several fundamental coenzymes of intermediate metabolism and plant photosynthesis (CoA, DPN, TPN, FAD) are nucleoside phosphates. All these molecules contain purines or pyrimidines which have strong ultra-violet absorption maxima near 2600 Å. The possibility then arises that the absorption of ultra-violet photons by purines and pyrimidines provided the bond energy for the synthesis of nucleoside phosphates in primitive times; and it is therefore of some interest to investigate the ultra-violet transparency of the early terrestrial atmosphere.

There is evidence from astronomy[8,9] that the Earth's atmosphere was reducing at the time life first arose. Laboratory experiments have shown that it is far easier to synthesize organic matter under reducing than under oxidizing conditions[10–12]. The molecules O_2 and O_3 are thermodynamically unstable in an excess of hydrogen. and the principal sources of the ultra-violet opacity of the present terrestrial atmosphere cannot have then been present. The ultra-violet absorption which did exist arose from intermediate oxidation state molecules. principally aldehydes and ketones. In experiments in which electrical discharges were passed through simulated primitive atmospheres, the only aldehyde or ketone produced in high yield was formaldehyde[13]. Nevertheless, the production of some acetaldehyde[14] and acetone can be expected. Formaldehyde absorption extends longwards of about 2900 Å. Acetaldehyde and acetone absorb throughout the 2400–2900 Å region. Ammonia, acetylene, and other molecules absorb shortwards of 2400 Å.

Therefore, the question of the transparency of the primitive terrestrial atmosphere near 2600 Å turns mainly on the unknown early abundance of CH_3CHO and CH_3COCH_3. Because of the relatively low acetaldehyde and acetone yields in simulation experiments, and because of possible independent biological indications of high ultra-violet fluxes in primitive times[2], it seems likely that the early reducing atmosphere was at least slightly transparent between 2400 and 2900 Å. From models of the evolution of the Sun, and an integration of the Planck function, the ultra-violet flux of wave-length $2900 \text{ Å} \geqslant \lambda \geqslant 2400 \text{ Å}$ incident on the Earth's atmosphere 4×10^9 years ago is computed to be about 7×10^{14} photons cm^{-2} sec^{-1} (ref. 15). Even with substantial atmospheric absorption, ultra-violet radiation in this window will greatly exceed other energy sources for organic synthesis[16].

The synthesis of purines and pyrimidines which absorb in this wave-length region has recently been accomplished in a variety of primitive Earth simulation experiments. Adenine has been produced by thermal polymerization

of 1·5 molar hydrocyanic acid in an aqueous ammonia solution[17]; by 5-MeV electron irradiation of methane, ammonia, water and hydrogen[18]; and by ultra-violet irradiation of a 10^{-4} molar solution of hydrocyanic acid[19]. Guanine also appears to be formed in the last experiment. Another guanine synthesis occurs in the thermal copolymerization of amino-acids[20]. Uracil has been produced by heating urea and malic acid[21].

The yields of purines and pyrimidines are sometimes quite high. In the electron beam irradiation of primitive atmospheres by Ponnamperuma, Lemmon, Mariner and Calvin[18], autoradiography indicates that the substance produced in highest yield is adenine. Thus it appears possible that ultra-violet light passing the 2400–2900 Å partial window in the primitive terrestrial atmosphere was strongly absorbed by purines and pyrimidines in the early oceans.

The production rates of organic molecules from reducing atmospheres suggest that the primitive oceans were about a 1 per cent solution of organic matter[2,9]. In addition to purines and pyrimidines the pentose sugars, ribose and 2-deoxyribose, can be expected to be present. The laboratory production of 2-deoxyribose has been achieved through the condensation of formaldehyde and acetaldehyde, or of acetaldehyde and glyceraldehyde in aqueous salt solutions[22]. (Indeed, this is an example of a mechanism which keeps the atmospheric aldehyde concentration low.) Both ribose and 2-deoxyribose have been synthesized by either ultra-violet or γ-irradiation of dilute formaldehyde solutions[23]. Phosphates and other phosphorus compounds can be expected in the primitive oceans, even at very early times[24].

It therefore seems of some interest to attempt synthesis of nucleoside phosphates by ultra-violet irradiation of dilute solutions of purine or pyrimidine bases, pentose sugars, and phosphorus compounds, both because of our expectation that such syntheses were easily performed in primitive times, and because ultra-violet irradiation of dilute solutions of adenine and ribose has already produced the nucleoside adenosine[25].

Materials and Experimental Techniques

Adenine-8-^{14}C of specific activity 23·4 μc./mg, adenosine-8-^{14}C of specific activity 7·2 μc./mg, and adenylic acid-8-^{14}C of specific activity 3·1 μc./mg were supplied by Schwarz Bioresearch, Orangeburg, New York. The non-radioactive AMP, ADP and ATP used as carriers were supplied by C. F. Boehringer, Mannheim, Germany. The adenosine tetraphosphate was a gift of Dr. John Moffatt of Syntex, Ltd., Palo Alto, California.

The ethyl metaphosphate used in the experiment was prepared by dissolving 150 gm of phosphorus pentoxide in

Table 1

Exp.	Adenosine	AMP	ADP	ATP	A4P
(1)					
(i) Adenine-^{14}C + ribose					
(ii) Adenine-^{14}C + ribose + phosphoric acid	+ (0·01%)	−	−	−	−
(iii) Adenine-^{14}C + ribose + ethyl metaphosphate	+ (0·01%)	+ (0·08%)	+ (0·06%)	+ (0·05%)	+ (0·04%)
(2)					
(i) Adenosine-^{14}C + phosphoric acid		−	−	−	−
(ii) Adenosine-^{14}C + ethyl metaphosphate		+ (0·5%)	+ (0·2%)	+ (0·1%)	+
(3)					
(i) Adenosine monophosphate-^{14}C + phosphoric acid			−	−	−
(ii) Adenosine monophosphate-^{14}C + ethyl metaphosphate			+ (3%)	+ (0·3%)	+ (0·1%)
(4)					
(i) Adenosine diphosphate + phosphoric acid				−	−
(ii) Adenosine diphosphate + ethyl metaphosphate				+	+

Figures in brackets show conversion as percentage of starting material. With the techniques used in this experiment the lower limit of detectability was 0·001 per cent.

In Exp. 4 no quantitative estimates were performed, as unlabelled ADP was used. The ATP in this case was located by shadowgrams.

300 ml. of ethyl ether and refluxing the solution for several hours with chloroform[26]. The excess solvent was removed by evaporation under vacuum, leaving a syrupy residue of ethyl metaphosphate.

The method of irradiation and analysis has already been described[25]. Quantities of the labelled adenine, adenosine and adenylic acid, varying from $1{\cdot}5 \times 10^{-6}$ to $1{\cdot}5 \times 10^{-5}$ moles in various experiments, were sealed in aqueous solution in 'Vycor' tubes with approximately stoichiometric quantities of ribose, phosphoric acid or polyphosphate ester, as shown in Table 1. The final concentration of base, nucleoside and nucleotide in each solution did not exceed 10^{-3} moles/l. The solutions were irradiated by four General Electric ultra-violet germicidal lamps, type 782*H*-10, which emit 95 per cent of their light in the mercury resonance line at 2537 Å. The 'Vycor' glass of which the tubes were made transmitted 80 per cent of the light incident at this wave-length. During a 1-h irradiation, the sample absorbed a total of $\sim 10^8$ ergs. During the irradiation the ambient temperature of the samples was $40° \pm 2°$ C.

The reaction products were first analysed by paper chromatography, autoradiography and ultra-violet absorption studies. An aliquot of the reaction products was spotted on a Whatman No. 4 paper and the chromatogram run in two solvents, butanol–propionic acid–water[27] and isobutyric acid–ammonia[28]. The positions of the carrier adenosine, AMP, ADP, ATP and A4P were detected by shadowgrams[29]. Coincidence both in position and in shape between the carriers on the shadowgrams and the radioactivity on the autoradiograph was the chromato-

graphic basis for the identifications. The formation of adenosine has already been reported[25] A further aliquot was chromatographed in two other solvent systems, trichloroacetic acid–acetone[30] and butanol–formic acid–water[31]. Once again there was coincidence between the carrier as outlined in the shadowgram and the radioactivity on the film.

Separations effected using thin-layer chromatography, and ion-exchange chromatography confirmed the results obtained from paper chromatography.

Results

The results of the investigation are summarized in Table 1 and in Figs. 1–4. Four different categories of experiments were performed. In the first the starting material was adenine, in the second adenosine, in the third adenosine monophosphate, and in the fourth adenosine diphosphate. The conversion of adenine to adenosine,

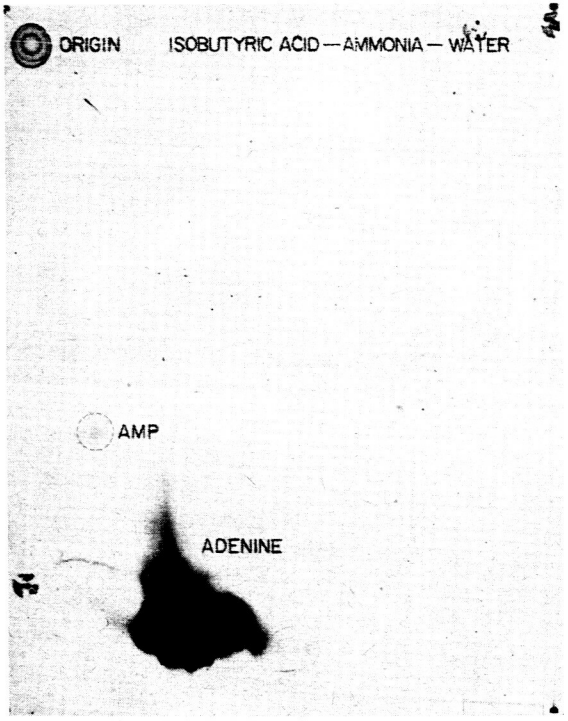

Fig. 1. Autoradiogram illustrating the formation of AMP from adenine, ribose and ethyl metaphosphate by the action of ultra-violet light

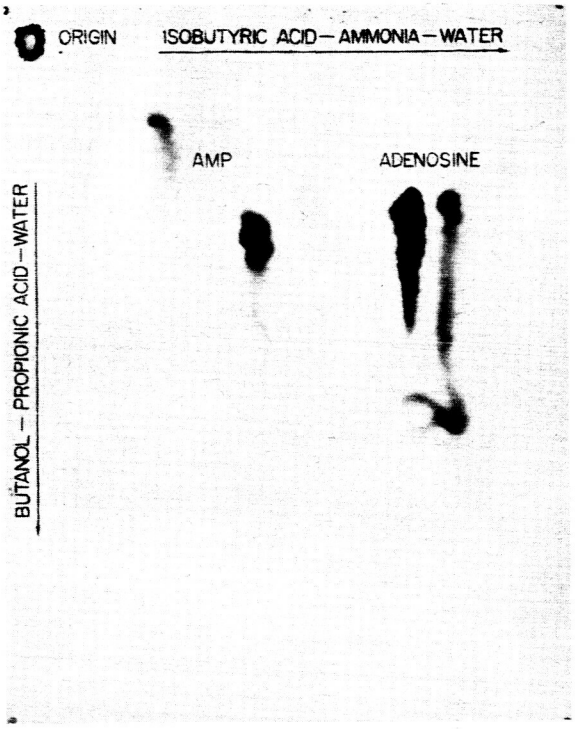

Fig. 2. Autoradiogram illustrating the formation of AMP from adenosine, and ethyl metaphosphate by the action of ultra-violet light. The long feature to the right of the teardrop-shaped adenosine spot is adenine, produced from adenosine photolysis. The dark central feature between AMP and adenosine is at present unidentified

adenosine to adenosine monophosphate, adenosine monophosphate to adenosine diphosphate, and adenosine diphosphate to adenosine triphosphate has been established. Experiments using adenine as the starting material have produced adenosine, AMP, ADP, ATP and A4P.

The previously reported experiment showed that adenosine is not produced in detectable amounts in the absence of a phosphorus compound[25]. While adenosine is produced in the presence of both phosphoric acid and ethyl metaphosphate, the nucleoside phosphates were detected only with the use of ethyl metaphosphate. Phosphoric acid was chosen first in the attempt to synthesize the nucleoside phosphates. Ethyl metaphosphate was selected as a possible reagent because of a recent report[26] that it activates carbonyl, hydroxyl and amino groups in organic synthesis. Other phosphorus compounds may also be effective in this synthesis, but they have not yet been investigated.

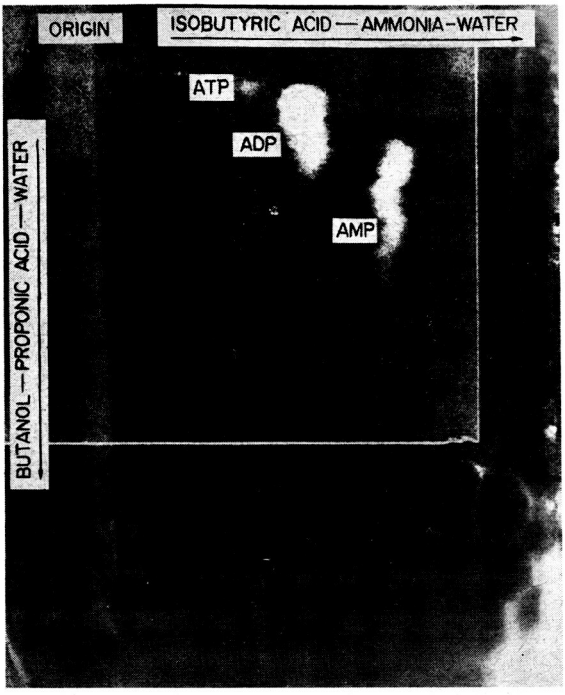

Fig. 4. Shadowgram illustrating the formation of ATP from ADP and ethyl metaphosphate by the action of ultra-violet light. The AMP is a photolytic product

Discussion

The abiogenic non-enzymatic production of nucleoside phosphates and related molecules under simulated primitive Earth conditions is relevant to the problem of the origin of life. The expected availability of ATP in primitive times suggests that energy was then available in convenient form for endergonic synthetic reactions of large molecules. The question arises why adenosine triphosphate, rather than, for example, the triphosphates of guanosine, cytidine, uridine, or thymidine, were not produced in primitive times and utilized to-day as the primary biological energy currency. There are several possible responses. In primitive Earth simulation experiments under reducing conditions with low hydrogen content, adenine is produced in far greater yield than are other purines and pyrimidines[17–19]. Secondly, no biological purine or pyrimidine has a larger absorption cross-section between 2400 and 2900 Å. Thirdly, adenine is among the most stable of such molecules under ultra-violet irradiation. Finally, the ultra-violet excitation

energy is readily transferred, especially by π electrons, along the conjugated double bonds of the molecule; the excited states are very long-lived, and thereby serve to provide bond energies for higher synthetic reaction. All but the first of these properties of adenine derive from the fact that it has the greatest resonance energy of all the biochemical purines and pyrimidines[32,33]. It thus appears that molecules ideally suited for the origin of life were preferentially produced in primitive times.

The yields achieved in these experiments, as shown in Table 1, are relatively quite high. In contrast, quite elaborate methods are ordinarily required for the laboratory synthesis of nucleoside phosphates[34]. For the production of adenosine from adenine, ribose and a phosphorus source, the quantum yield for a 1-h irradiation is $\varphi \sim 10^{-5}$. For production of AMP, ADP and ATP by the use of ethyl metaphosphate, the quantum yields are almost an order of magnitude greater.

It is not now known to what extent the experiments here reported accurately reproduce the environmental conditions in the primitive terrestrial oceans. It can be expected that ethyl metaphosphate was probably not the most abundant phosphorus source; but we do not know how well other, possibly more abundant, phosphate salts may efficiently substitute for ethyl metaphosphate. The irradiation period in these experiments was 1 h. Continued irradiation, with no removal of products, must, by the second law of thermodynamics, ultimately result in lower over-all quantum yields. The influence of inorganic anions on the course and rate of these reactions is largely unknown. Nevertheless, it is of some heuristic interest to compute the production rate of adenosine triphosphate in the primitive terrestrial oceans, were the conditions there similar to those in the present experiments.

The production rate of ATP in the primitive reducing atmosphere will then be:

$$\frac{d\sigma}{dt} \simeq \frac{Q\varphi\mu}{4N_A} \text{ g cm}^{-2} \text{ sec}^{-1}$$

where Q is the ultra-violet photon flux for 2400 Å $\leqslant \lambda \leqslant$ 2900 Å, φ is the quantum yield, μ is the molecular weight of ATP, and N_A is Avogadro's number[15]. Taking $Q \sim 7 \times 10^{14}$ photons cm^{-2} sec^{-1} (ref. 15) $\varphi \sim 3 \times 10^{-5}$, and $\mu \sim 550$, we derive:

$$\frac{d\sigma}{dt} \simeq 5 \times 10^{-12} \text{ g cm}^{-2} \text{ sec}^{-1}$$

A feeling for the magnitude of this figure can be obtained by computing the steady-state population of microorganisms over the entire globe that could be maintained by this quantity of abiologically produced adenosine triphosphate. That is, we assume that the primitive Earth is populated by obligate heterotrophs that obtain all their energy from abiologically synthesized ATP[5]. We

will obtain a mimimum population if we assume that the number of ATP molecules required for each replication and the doubling time per cell have values characteristic of typical contemporary organisms. Taking values for *Eschericia coli* of 10^9 ATP molecules per cell for each doubling, and a doubling time of 1 h, we find the required ATP production rate to maintain one cell must be $2{\cdot}5 \times 10^{-16}$ g sec^{-1} $cell^{-1}$. The steady-state population of micro-organisms that can be maintained over the entire globe by the abiological synthesis of ATP is then 2×10^4 cells/cm^2 column of ocean. This estimate is, of course, extremely approximate. The assumptions that all the ultra-violet light is transmitted by the atmosphere, that it is all absorbed by adenine in the ocean and that the quantum yields used in the ethyl metaphosphate experiments are applicable to the primitive environment probably increase the derived steady-state cell population; while the assumptions that the ATP requirement and doubling time for primitive organisms are the same as for *E. coli* probably decrease the derived steady-state cell poulation over the true value. Nevertheless, this calculation does suggest that abiogenic ATP production by ultra-violet light in primitive times may have supported quite sizable populations of micro-organisms on the primitive Earth.

Such abiogenic production of ATP is, in effect, photosynthesis without life. One striking conclusion that has emerged from recent work on the mechanism of terrestrial plant photosynthesis is that the production of ATP is the primary, and most primitive, function of the photosynthetic apparatus[35,36]. The experimental results of the present article permit us to understand why this might be so. With rather efficient abiogenic synthesis of so ideal an energy currency as ATP in the primitive environment, the transition from a reducing to an oxidizing atmosphere must have had profound results.

The transition was at least partially initiated by the ultra-violet photodissociation of water vapour in the high atmosphere, and the selective escape of hydrogen to space[8,37]. The ozone concentration of a planetary atmosphere depends approximately logarithmically on the oxygen concentration, down to a certain lower limit of the oxygen concentration[38,39]; thus the steady-state production of even 10^{-4} or 10^{-5} of the present oxygen concentration would have produced enough ozone to diminish the ultra-violet flux in the 2400–2900 Å partial window, and make the rate of ultra-violet synthesis of ATP decline. A premium was then placed on organisms with the ability to utilize visible light for ATP synthesis. One can imagine the metabolism of the primitive organisms to be so keyed to the availability of ATP that the first visible photosynthetic apparatus evolved would be adopted by all subsequent life forms.

The precise mechanism of synthesis has not yet been

investigated. Ultra-violet excitation of adenine accounts for the adenosine synthesis, but the participation of phosphorus compounds in the reaction is obscure. Synthesis of nucleoside phosphates must be more indirect, since it is difficult to imagine the excitation energy being transferred across the ribose molecule, which has no conjugated double bonds. Alternative possibilities, such as the production of activated adenine or ribose phosphates, remain to be investigated.

Further investigation of so far unidentified chromatographic features should both help clarify the mechanisms of synthesis and cast light on other possible prebiological organic reactions. Ultra-violet irradiation of solutions of deoxyribose, purines or pyrimidines, and phosphate compounds may have some relevance for the problem of polynucleotide origins.

Bio-assay. To establish whether the ATP synthesized by us was biochemically active, a luminescence assay was performed using dehydrated firefly tails[40]. The method described by Strehler and Trotter was used. (Firefly tails were supplied by Schwarz Bioresearch, Inc., Mount Vernon, New York.) The intensity of luminescence was measured by a Turner fluorometer. The decay curve of the luminescence was identical with that of an authentic sample of ATP. The concentration of ATP in the solution used, as determined by this method, corresponded within the limits of experimental error to the value obtained by spectrophotometric measurements.

The synthetic ATP was further tested for biochemical activity by Roberta Kupervas and Dr. Harold Klein of the Exobiology Division of Ames Research Center, using yeast hexokinase. The synthetic product participated in this reaction in a manner equivalent to authentic ATP (Sigma Chemical Co., St. Louis, Mo.).

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